

CLAIMS

1. Staphylokinase derivatives showing a reduced immunogenicity as compared to wild-type staphylokinase, after administration to patients with arterial thrombosis.
2. Staphylokinase derivatives as claimed in claim 1 having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by another amino acid thus reducing the reactivity with a panel of murine monoclonal antibodies.
3. Staphylokinase derivatives as claimed in claim 1 having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by another amino acid thus reducing the absorption of SakSTAR-specific antibodies from plasma of patients treated with staphylokinase.
4. Staphylokinase derivatives as claimed in claim 1 having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by other amino acids, without reducing the specific activity by more than 50 percent.
5. Staphylokinase derivatives SakSTAR(K35X,G36X,E65X,K74X,E80X,D82X,K102X,E108X,K109X,K121X,K130X, K135X,K136X,+137X) having the amino acid sequence as depicted in figure 1 in which the amino acids Lys in position 35, Gly in position 36, Glu in position 65, Lys in position 74, Glu in position 80, Asp in position 82, Lys in position 102, Glu in position 108, Lys in position 109, Lys in position 121, Lys in position 130, Lys in position 135 and/or Lys in position 136 have been replaced with other amino acids and/or in which one amino acid has been added at the COOH-terminus, thus altering the immunogenicity after administration in patients, without markedly reducing the specific activity.
6. Staphylokinase derivatives listed in Tables 1,3,4,5,6,7 and 8, having the amino acid sequence as depicted in figure 1 in which the indicated amino acids have been replaced by

other amino acids thus reducing the absorption of SakSTAR-specific antibodies from plasma of patients treated with staphylokinase, without reducing the specific activity.

7. Staphylokinase derivatives having an amino acid substituted with Cys, resulting in dimerization, increased specific activity, delayed clearance and/or increased thrombolytic potency.
8. Staphylokinase derivatives with polyethylene glycol substitution....
9. Method for producing the staphylokinase derivatives as claimed in claims 1 to 7, comprising the steps of:
 - a. preparing a DNA fragment comprising at least the part of the coding sequence of staphylokinase that provides for its biological activity;
 - b. performing in vitro site-directed mutagenesis on the DNA fragment to replace one or more codons for wild-type amino acids by a codon for another amino acid;
 - c. cloning the mutated DNA fragment in a suitable vector;
 - d. transforming or transfecting a suitable host cell with the vector; and
 - e. culturing the host cell under conditions suitable for expressing the DNA fragment.
10. Method as claimed in claim 9, wherein the DNA fragment is a 453 bp *EcoRI-HindIII* fragment of the plasmid *pMEX602sakB*, the *in vitro* site-directed mutagenesis is performed and the mutated DNA fragment is expressed in *E. coli*.
11. Pharmaceutical composition comprising at least one of the staphylokinase derivatives as claimed in claims 1 to 8 together with a suitable excipient.
12. Pharmaceutical composition as claimed in claim 11 for treating arterial thrombosis.